Abnormal intracellular Ca^{2+} cycling plays a key role in cardiac dysfunction, particularly during the setting of cardiac ischaemia/reperfusion (I/R). At the onset of reperfusion there is an increase in cytosolic Ca^{2+}, which has been usually associated with an uncontrolled influx from the extracellular space. Ca^{2+} mishandling has been in turn related to reperfusion arrhythmias. However, the basic mechanism for these arrhythmias is still unresolved. We performed experiments to test the hypothesis that the increase in cytosolic Ca^{2+} at the onset of reperfusion originates in Ca^{2+} efflux from the sarcoplasmic reticulum (SR) and propagates through the cell as cytosolic Ca^{2+} waves serving as a trigger for the initiation of reperfusion arrhythmias. By using a combination of pulsed local-field fluorescence (PLFF) and laser scanning confocal microscopy in mouse intact hearts loaded with Rhod-2 and/or Mag-Fluo-4 (cytosolic and/or SR Ca^{2+} measurements) or Fluo-4 (Ca^{2+} sparks and waves) and submitted to global I/R (12/30 min), it was found that ischemia evoked an increase in cytosolic and SR Ca^{2+}. This increase was associated with a significant rise in Ca^{2+} sparks relative to preischemia: 2.07 ± 0.33 vs. 1.13 ± 0.20 sp/sec/100µm ($P<0.05$, ANOVA). Reperfusion evoked an increment in cytosolic Ca^{2+} (Ca^{2+} *bump*) that was associated with a significant decrease in SR Ca^{2+} and in Ca^{2+} sparks with respect to ischemia and a significant increase in Ca^{2+} waves relative to ischemia and preischemia: 0.71 ± 0.14 vs. 0.38 ± 0.06 and 0.25 ± 0.04 w/sec/100µm. The results show the first direct evidence of an increase in Ca^{2+} sparks in ischemia that transform in Ca^{2+} waves during reperfusion. These waves may constitute a main trigger of reperfusion arrhythmias. Supported by NIH R01-HL-084487 and PICT/1903 (FONCYT).

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Oxidation of Ryanodine Receptor following Ischemia/Reperfusion Increases the Propensity of Ca Waves during Beta-Adrenergic Receptor Stimulation

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Beta-Adrenergic receptor (beta-AR) stimulation generates the main positive inotropic response in the heart. However, after ischemia/reperfusion (I/R), beta-AR stimulation can generate arrhythmias. I/R also increased intracellular reactive oxygen species (ROS) production. We investigated whether ryanodine receptor (RyR) oxidation by ROS contributes to the transition from positive inotropic to arrythmogenic effect in ventricular myocytes after I/R. Measurements of contractile and electrical activity from ex vivo rabbit hearts revealed that global I/R produces severe tachi-arrhythmias. Ventricular myocytes isolated from ischemic hearts were characterized by increased both SR Ca leak rates and fractional SR Ca release compared to cells from non-ischemic hearts. Furthermore, myocytes from ischemic hearts showed increased ROS production, decreased level of free thiols in RyRs (RyR oxidation), and increased level of oxidized glutathione (GSSG). Pretreatment of myocytes from ischemic hearts with the reducing agent mercaptopropionylglycine attenuated the oxidization of free thiols in RyRs and normalized systolic SR Ca release and diastolic Ca leak. In myocytes from ischemic hearts, isoproterenol (ISO; 10 nM) led to the occurrence of spontaneous Ca waves, whereas in cells from non-ischemic hearts the same dose of ISO only caused a positive inotropic effect. Treatment of myocytes from non-ischemic hearts with H2O2 (0.1 mM) increased SR Ca leak and fractional SR Ca release to similar levels observed in myocytes from ischemic hearts. Moreover, application of ISO (10 nM) to myocytes from non-ischemic hearts pretreated with H2O2 increased the propensity of Ca waves. These results indicate that augmentation of ROS production following I/R causes RyR oxidation. This post-translational modification of the RyR plays a critical role in the transition from positive inotropic to arrhythmogenic effect during beta-AR stimulation.

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Extracellular Cathepsin-L Alters Ca^{2+} Transient Amplitude and Sarcoplasmic Reticulum Ca^{2+} Content in Adult Rat Cardiomyocytes

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Serum levels of Cathepsin-L (CatL), a ubiquitous cysteine protease, are increased in patients with ischaemic heart disease. It is unknown whether CatL could (i) be released by the heart following ischaemia and (ii) contribute to altered sarcoplasmic reticulum (SR)-mediated function in cardiomyocytes during cardiac disease. *Ex vivo* perfused rat hearts underwent 30min global ischaemia with subsequent reperfusion. CatL activity was detected (Z-LR-AMC fluorometric assay) in coronary effluent throughout the 90 min post-reperfusion period. The effect of extracellular CatL on SR-mediated Ca^{2+} release across a range of reported concentrations found in patients with coronary heart disease was also determined. Ventricular rat cardiomyocytes were isolated, loaded with Fura-2AM and incubated (30min) with recombinant CatL/vehicle at 0.68nM(n=23), 2.70nM(n=22) and 5.40nM (n=18). Cardiomyocytes were field-stimulated (0.5Hz) and perfused with a modified Krebs-Henseleit solution containing CatL/vehicle. CatL activity at physiological pH within the perfusate was confirmed. using epifluorescence microscopy, Ca^{2+} transient parameters during field-stimulation and during rapid application of 10mM caffeine were determined. Ca^{2+} transient amplitude was decreased in a concentration-dependent manner by the above concentrations of CatL (Control:100 ± 4.6 (n=67) *vs.*79.8 ± 7.1 (0.68nM), 68.8 ± 4.8 (2.7nM), 42.9 ± 5.2% (5.4nM); $P<0.05$) *via* reduced transient peak $[Ca^{2+}]_i$ (Control:100 ± 4.2 *vs.*80.6 ± 6.5, 73.0 ± 4.3, 53.6 ± 5.0%; $P<0.05$). The *tau* of Ca^{2+} transient decay was prolonged at 2.7 and 5.4nM (Control:0.9 ± 0.1 *vs.* 1.5 ± 0.1, 2.4 ± 0.2s; $P<0.05$), but not at 0.68nM. The caffeine-induced Ca^{2+} transient amplitude was reduced (Control:100 ± 5.1 *vs.* 78.8 ± 5.0, 81.5 ± 5.2, 60.1 ± 8.5%; $P<0.05$) however *tau* was not significantly affected (1.4 ± 0.1 *vs.* 1.3 ± 0.1s, 1.4 ± 0.1, 1.7 ± 0.1, 1.1 ± 0.1; $P>0.05$). This study demonstrates that CatL is released from ischaemic hearts upon reperfusion and depresses SR-mediated Ca^{2+} release in field-stimulated Ca^{2+} transients in a concentration-dependent manner. Extracellular CatL may therefore have the potential to contribute to cardiac dysfunction in patients with heart failure.

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Exploring the Link between Ca^{2+} Signalling and ATP Dynamics in Cardiomyocyte Dysfunction

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In heart failure the progressive deterioration of cardiac function is linked to Ca^{2+} handling abnormalities, altered metabolism and increased cardiomyocyte apoptosis. However the causal versus consequential nature of these linked phenomena remains to be determined. Here we aimed to investigate cellular ATP and Ca^{2+} environments and necrotic and apoptotic cell death in cellular models of Ca^{2+} dysfunction and metabolic perturbation. We constructed new ATP sensors via fusion of a red fluorescent protein (mCherry) with the pH-independent click-beetle luciferase in both mCherry-Luc and Luc-mCherry configurations. Chimeric proteins exhibited high specific activities of the luciferase partner in vitro (approximately 5 cps/amol protein) and we determined linear fluorescence-luminescence relationships ($r2 > 0.88$) following their expression in COS-7 cells. The dynamic range of the Luc-mCherry configuration was determined via exposure of COS-7 cells to manoeuvres that depleted ATP (e.g. 2-DOG, FCCP) and under conditions of elevated glucose availability (> 6g/L extracellular). In HL-1 cardiomyocyte monolayers, we monitored cellular ATP environments following pharmacologic manipulation of the amplitude and temporal patterning of Ca^{2+} signaling (e.g. using ouabain, staurosporine, BAPTA). Ca^{2+} and ATP environments were monitored through the transitions from spontaneously oscillatory syncytia to non-oscillatory phenotypes. Under these conditions, we measured cellular ATP effluxes and susceptibility to apoptosis using in situ DNA fragmentation and caspase activation assays. This approach reconciles specific perturbations in Ca^{2+} handling, the cellular ATP environment and phenotypic outcomes and builds a more complete picture of the events that contribute to the early stages of cellular dysfunction in chronic heart disease.

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Dissecting the Calcium Transient Refractoriness in Mouse Ventricular Myocytes

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The activation cascade of Ca^{2+} induced Ca^{2+} release (CICR) to produce a $[Ca^{2+}]_i$ transient has been well described; on the other hand, mechanisms responsible for its termination and refractoriness are still a matter of debate. It is known that the ryanodine receptor (RyR) state and sarcoplasmic reticulum Ca^{2+} content ($[Ca^{2+}]_{SR}$) are major determinates in the termination and refractoriness of Ca^{2+} sparks at room temperature. The goal of this work was to evaluate $[Ca^{2+}]_i$ transient refractoriness (CaTR) when RyR state and/or $[Ca^{2+}]_{SR}$ was altered by pharmacological means at physiological temperature (36 ± 1°C). CaTR was measured in mouse ventricular myocytes loaded with Fluo-5F and paced at 2Hz (field stimulation or depolarization through patch pipette) followed by an extra-stimuli with a decreased interval (ESI). This enabled us to measure a $[Ca^{2+}]_i$ transient restitution curves. The $[Ca^{2+}]_i$ transient peak recovered exponentially as ESI increased ($\tau \sim 137$ms). L-type Ca^{2+}